Environ Health Perspect
DOI: 10.1289/EHP876

Supplemental Material

Effect of Coal Fly Ash Particulate Matter on the Antimicrobial Activity of Airway Surface Liquid

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by *t* test, C was compared to inoculum by multiple comparison test ANOVA. * $p < 0.05$, ** $p < 0.001$).

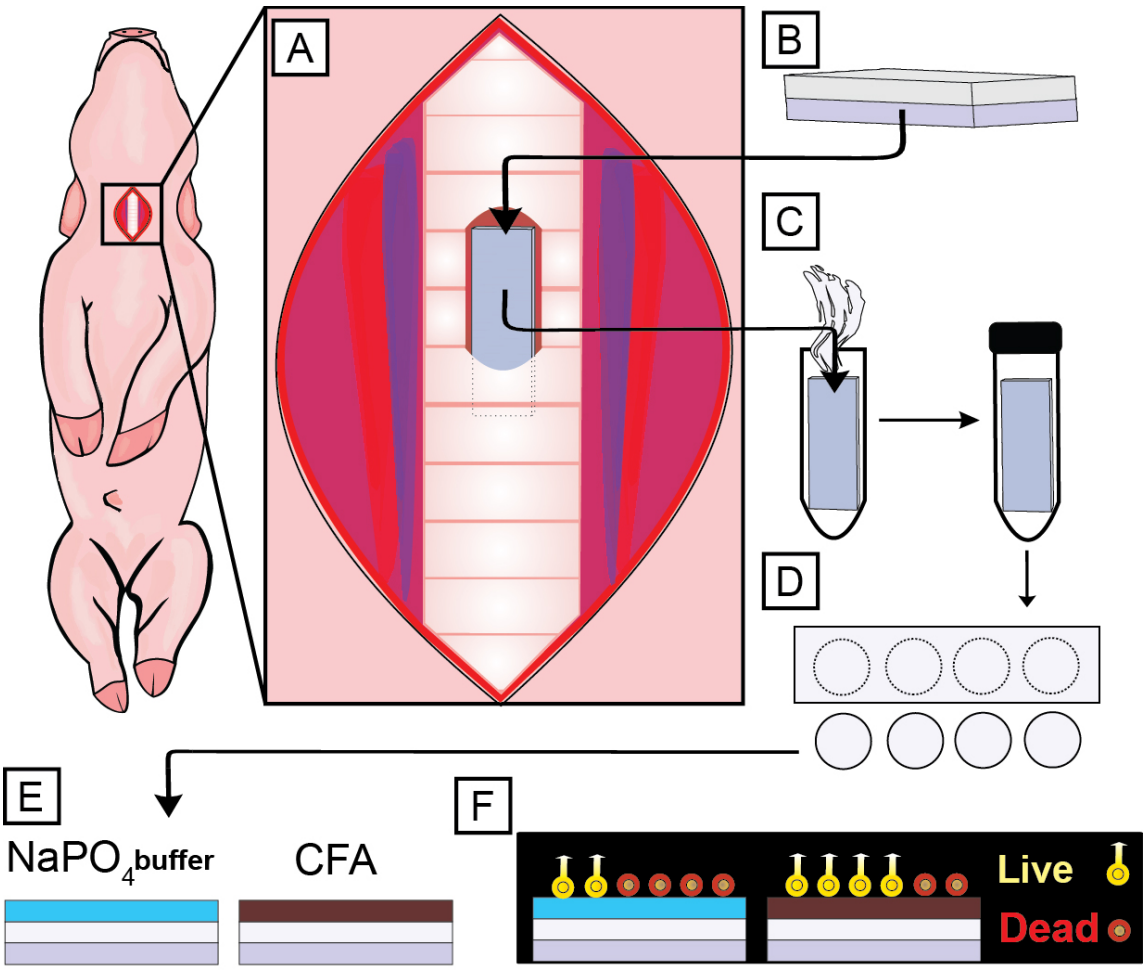

Fig. S1. Pig *ex-vivo* ASL collection. (**A**) Trachea was surgically exposed. (**B**) Parafilm fused to lens paper was placed on the tracheal lumen for 30 seconds, and extracted avoiding damage to the trachea. (**C**) Samples were dehydrated overnight and stored at -80°C. (**D**) Strips were cut with a 4 mm biopsy punch the day of the experiment. (**E**) Samples were rehydrated with buffer or CFA solution for 1 hour at 37 °C. (**F**) Bioluminescent bacteria were injected onto the paper with the ASL sample and RLU were measured after 2 min.

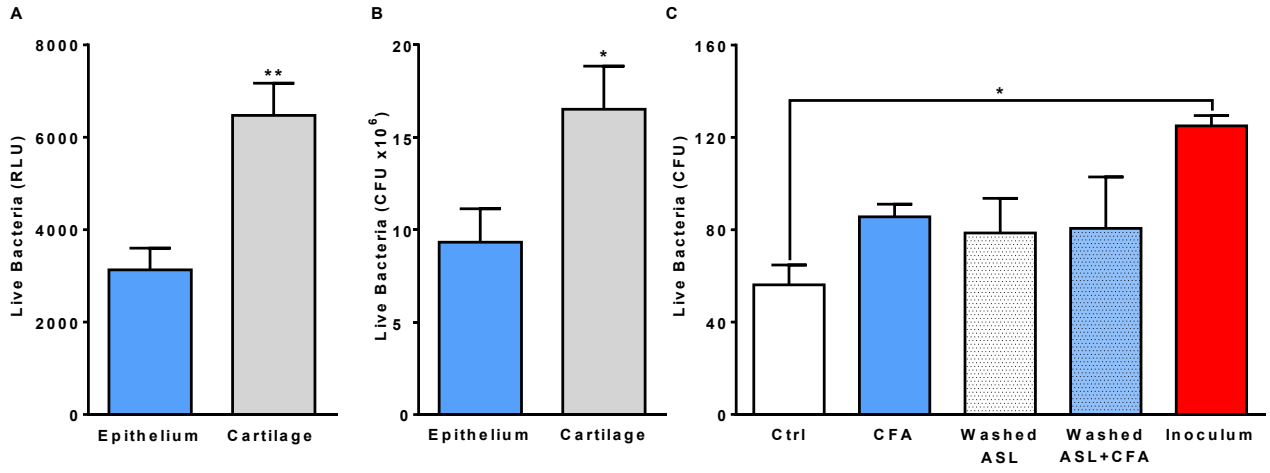

Fig. S2. CFA impairs airway explant ASL antimicrobial activity. (**A**) Pig airway explant antimicrobial activity assessed by RLU with and without epithelium (n=7). (**B**) Pig airway explant antimicrobial activity assessed by CFU counting with and without epithelium (n=7). When we denuded the epithelium out of the nasal septum explants, we observed a significant increase in live bacteria compared to the intact sample. (**C**) Effect of exposure to CFA, and ASL washing, on pig nasal epithelium explant antimicrobial activity. Only intact epithelia, exposed to buffer control, significantly decreased the inoculum of bacteria administered. When we exposed airway (diameter 2 mm^2) to CFA (3µg/cm^2), we observed impaired antimicrobial activity similar to epithelia whose ASL had been washed away with PBS-/-. Exposure to CFA, in the epithelia without ASL, does not increase bacteria at 20 min (n=7). (A and B, were compared to epithelium by *t* test, C was compared to inoculum by multiple comparison test ANOVA. * $p< 0.05$, ** $p< 0.001$).